Journal of the American Mosquito Control Association, 20(3):239–242, 2004

THE EFFECTIVENESS OF LIGHT, 1-OCTEN-3-OL, AND CARBON DIOXIDE AS ATTRACTANTS FOR ANOPHELINE MOSQUITOES IN MADANG PROVINCE, PAPUA NEW GUINEA

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ABSTRACT. The effectiveness of light, 1-octen-3-ol (octenol), carbon dioxide (CO_2) and a combination of CO_2 and octenol were compared as mosquito attractants using encephalitis vector surveillance traps in 2 villages in Madang Province, Papua New Guinea (PNG). Five species were collected, *Anopheles koliensis, Anopheles farauti* 2, *Anopheles farauti* 4, *Anopheles longirostris,* and *Anopheles bancroftii.* Light alone was not attractive to any of these species, and the attractiveness of octenol alone, though greater than light, was less than that of CO_2 or the CO_2 + octenol combination. With *An. longirostris,* the addition of octenol to CO_2 resulted in a statistically significant increase in trap numbers; however, for the other species, any increase was not significant, and with *An. koliensis* and *An. bancroftii,* trap numbers were actually reduced when the CO_2 + octenol bait was used. In PNG, the use of octenol alone would be effective in attracting more anophelines than if light alone was used; however, octenol by itself was not as effective as CO_2.

KEY WORDS Anophelines, attractants, octenol, Papua New Guinea

INTRODUCTION

Sampling of mosquito populations is essential for studies on disease transmission and the evaluation of control measures. The most widely used methods for collecting mosquitoes have been those using human and animal baits. However, over the last 10 years, there has been an increasing use of mosquito traps for making mosquito collections because these have the advantage of being less labor intensive and the human collectors are not exposed to infective bites. The effectiveness of these traps has been enhanced by using carbon dioxide (CO_2) as an attractant. Rudolf in 1922 (Service 1993) first reported the value of this compound as a mosquito attractant. Newhouse et al. (1966) showed that, when CO_2 was used with Centers for Disease Control (CDC) light traps, mosquito collections increased 4-fold and the number of species collected increased by 20–26%. The effectiveness of CO_2 as an attractant has led to the development of the encephalitis vector surveillance (EVS) trap (Rohe and Fall 1979). This trap incorporated the use of CO_2 in the form of dry ice held in an insulated container from which the rest of the trap was suspended. Carbon dioxide-baited mosquito traps are now one of the main collecting methods for studying the transmission of mosquito-borne diseases, particularly arboviruses.

Besides CO_2, numerous other compounds, such as butanone, honey extract, 1-octen-3-ol (octenol), L-lactic acid, and phenols have been evaluated as attractants for mosquitoes (Kline et al. 1990). Of these, octenol appears the most promising. Isolated from bovine breath, octenol was originally used as an attractant for tsetse flies (Vale and Hall 1985). Takken and Kline (1989) first demonstrated that it also acted as an attractant for mosquitoes, particularly when used in combination with CO_2. Since then, several studies have shown that this bait combination works well for increasing trap collections of various species of *Aedes* and *Ochlerotatus* but was less effective for *Culex* species and produced mixed results within and between different species of *Anopheles* (summarized in Kline 1994).

The major vectors of malaria in Papua New Guinea (PNG) belong to the *Anopheles punctulatus* Dönitz group (Burkot et al. 1988); however, recent studies have also incriminated *Anopheles bancroftii* Giles and *Anopheles longirostris* Brug as vectors (Hii et al. 2000). The *An. punctulatus* group consists of at least 12 species, 10 of which are found in PNG. Although morphological markers can identify some of these species, the 7 members of the *Anopheles farauti* Laveran complex within the group appear to be isomorphic (Cooper et al. 2002). Due to the medical importance of these anophelines, surveys have been conducted to study their role in malaria transmission, and these have relied primarily on human landing collections (Burkot et al. 1988, Hii et al. 2000). The difficulty of obtaining a reliable supply of dry ice in PNG has precluded the large-scale use of mosquito traps for this type of work. The purpose of this study was to observe the response of PNG anopheline species to traps baited with light, octenol, CO_2, and CO_2 + octenol and to determine if octenol could substitute for CO_2 as an attractant to increase trap collections.

MATERIALS AND METHODS

The study was conducted in the villages of Pumpres and Umun near Madang town on the north coast of Papua New Guinea. Pumpres and Umun

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Table 1. Mean (±SE) number of anopheline mosquitoes attracted to encephalitis virus surveillance traps using different baits at Umun and Pumpres villages in Madang Province, Papua New Guinea.[1]

Site	Species	Baits			
		Light	Octenol	CO_2	CO_2-octenol
Umun	*Anopheles koliensis*	0.5 ± 0.3 a	7.8 ± 4.2 b	21.3 ± 4.3 c	25.0 ± 6.1 c
(*n* = 4)	*An. farauti* s.l.[2]	0.0 ± 0.0 a	8.0 ± 4.3 b	10.5 ± 3.5 bc	23.8 ± 6.1 c
	An. bancroftii	1.0 ± 0.4 a	6.8 ± 3.5 b	13.8 ± 4.4 b	7.0 ± 1.8 b
	An. longirostris	0.0 ± 0.0 a	0.5 ± 0.3 a	5.3 ± 2.6 b	31.0 ± 7.6 c
Pumpres	*An. koliensis*	1.0 ± 0.4 a	1.6 ± 0.7 a	16.8 ± 7.7 b	3.1 ± 1.0 a
(*n* = 8)	*An. farauti* s.l.[3]	0.5 ± 0.4 a	1.9 ± 0.6 b	5.5 ± 2.1 b	4.1 ± 0.8 b
	An. longirostris	0.0 ± 0.0 a	0.6 ± 0.3 a	0.6 ± 0.4 a	8.1 ± 3.2 b

[1] Means in the same row followed by the same letter are not significantly different ($P > 0.05$); Student–Newmann–Kuels test applied to $\log(x + 1)$ transformed data.
[2] Made up of *An. farauti* 2 (88.1%) and *An. farauti* 4 (11.9%).
[3] Made up of *An. farauti* 2 (87.5%) and *An. farauti* 4 (12.5%).

are 1 and 15 km from the sea, respectively. The climate of the region is tropical monsoon with an annual rainfall of 3,500–4,000 mm. Collections were carried out during the end of the regions' wet season in April 1995.

Trap collections were made using standard EVS traps. The light source was supplied by a 1.5-V bulb; octenol, where used, was released from a wick that protruded 10 mm from a reservoir vial (the medium release rate vial as described by van Essen et al. 1994). The vial was placed directly above the trap entrance. The containers holding octenol and dry ice were weighed before and after each night's trapping. Four traps, each with 1 of the following baits: light, octenol, CO_2, and CO_2 + octenol, were placed in the village under the eves of houses approximately 1 m above the ground and 20 m apart. The traps were rotated through each position once in Umun (4 trap nights) and twice in Pumpres (8 trap nights).

Adults, collected in traps, were killed by freezing, then identified using the morphological key of Lee and Woodhill (1944). Specimens belonging to the *An. punctulatus* group were frozen in liquid nitrogen and further identification made using species-specific DNA probes and the squash blot isotopic method of Cooper et al. (1991) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the internal transcribed spacer region 2 using the method of Beebe and Saul (1995).

Trap collection data were transformed ($\log[x + 1]$), then subjected to a 2-way analysis of variance using SigmaStat® to separate treatment effects from the effect of trap position and collection night. For significant treatment effects, means were compared using a Student–Newman–Kuels test using SigmaStat (Fox et al. 1994).

RESULTS AND DISCUSSION

During the study, 1,034 anophelines were collected from all traps and the following species identified: *An. koliensis* Owen (439), *An. farauti* 2 (232), *An. farauti* 4 (32), *An. longirostris* (223) and *An. bancroftii* (114).

The use of DNA species-specific probes confirmed the morphological identification of *An. koliensis*; however, they failed to resolve the identity of the members of the *An. farauti* complex. Though *An. farauti* 4 was identified from Pumpres and Umun, most specimens could not be identified using the *An. farauti* 1, 2, or 3 probes and the data on this material were pooled under *An. farauti* s.l. It was subsequently determined by PCR-RFLP that the majority (>87%) of *An. farauti* s.l. specimens collected at Pumpres and Umun were *An. farauti* 2. The DNA probe sequence for *An. farauti* 2 was made from material collected in Australia and it appears that it does not hybridize with *An. farauti* 2 from northern PNG.

In the baited traps, the mean release rate of octenol was 9.5 ± 0.73 mg/h and CO_2 was 537.3 ± 27.5 ml/min. These release rates are comparable with those used by other workers (Kline et al. 1991, van Essen et al. 1994, Ritchie and Kline 1995, van den Hurk et al. 1997).

A statistical comparison of the attractiveness of the different trap baits used at Pumpres and Umun is shown in Table 1. Traps with light attracted none or very few anophelines. A similar response has been found by other workers, and it was only when light traps were placed close to a human bait source (i.e., inside houses next to sleeping inhabitants) were they effective in collecting anophelines (Davis et al. 1995, Hii et al. 1986). When traps with light have been placed outside or in uninhabited rooms, their ability to attract anophelines has been poor (Charlwood et al. 1984, 1986; Wilton 1975).

The response to the baits, octenol, CO_2, and CO_2 + octenol, varied with the species of anopheline and the location (Table 1). The attractiveness of octenol was significantly greater than light for *An. koliensis, An. bancroftii,* and *An. farauti* s.l. at Umun and for *An. farauti* s.l. at Pumpres. With all species, CO_2 attracted higher numbers than the octenol; however, this increase was only statistically significant for *An. koliensis* at Umun and Pumpres and

An. longirostris at Umun. The addition of octenol to CO_2 did not result in a significant increase in the numbers of *An. bancroftii* or *An. koliensis* collected. This bait combination actually resulted in reduced trap numbers of *An. bancroftii* at Umun and *An. koliensis* at Pumpres. The difference in behavior between the Umun and Pumpres populations of *An. koliensis* with regard to the attractiveness of the CO_2 + octenol bait may be due to the availability of alternative animal hosts. Pigs, dogs, and chickens are common in PNG villages, but their numbers can vary greatly among villages and their availability appears to influence anopheline host selection (Charlwood et al. 1985). Only with *An. longirostris* was there a synergistic effect with the CO_2 + octenol bait combination, and the Umun and Pumpres populations of this species showed a statistically significant increase in the numbers collected. *Anopheles longirostris,* though recorded as biting humans, is thought to be predominantly a zoophilic species (Charlwood et al. 1985), this may account for its attractiveness to the CO_2 + octenol combination. However, of all the species collected, *An. longirostris* was the least attracted to octenol alone and to CO_2 alone.

Kline et al. (1991) noted that of all the culicid genera studied for their response to octenol and CO_2, anophelines were the most variable both within and between species. Van den Hurk et al. (1997) found similar inconsistencies in the response of members of the *An. farauti* complex in Australia to these attractants. At one location, *An. farauti* s.l., *An. farauti* 1, and *An. farauti* 2 showed a statistically significant increase in CDC trap numbers when octenol was combined with CO_2. However, at other locations, trap numbers of *An. farauti* s.l. and *An. farauti* 2 were not significantly increased when the CO_2 + octenol combination was used. In our study, we also found inconsistencies with *An. farauti* s.l. There was a synergistic effect with the CO_2 + octenol bait with *An. farauti* s.l. (>88% *An. farauti* 2) at Umun, though the increase was not statistically significant. However, in Pumpres, there were fewer *An. farauti* s.l. (>87% *An. farauti* 2) attracted to the CO_2 + octenol bait than to CO_2 alone, though the numbers of *An. farauti* s.l. in this village were low.

The results of this study show that the addition of octenol to a mosquito trap would improve trap collections of PNG anophelines, and in PNG, where CO_2 is not readily available, there would be an advantage in using octenol. The attraction of anophelines to octenol might be enhanced further if the trap was located near some type of bait such as a sleeping person or an animal; this concept would need further study.

ACKNOWLEDGMENTS

This paper is published with the approval of the Director General of Defence Health Services. We acknowledge the support of the Papua New Guinea Institute of Medical Research facilities at Madang, PNG.

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